MERCURY ANALYSIS IN ENVIRONMENTAL SAMPLES

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FOREWORD

Although mercury levels in environmental samples have been well documented in most provinces in Canada, the Province of Alberta has only recently begun detailed analysis for mercury in sediments and fish. Therefore, it is timely to bring out a Methods Manual for standardized analysis of mercury in different matrices of environmental samples. This Manual describes procedures for sampling, preservation, storage and analysis of mercury in fish, sediment, suspended solids and water. Routine quality control procedures, reference to standard materials and inter-laboratory comparisons are discussed in detail. Environmental decisions should be based on reliable even if limited data. This will help to avoid costly and unnecessary compliance programs based on false-positive results. Also, it will minimize the risk of disregarding potential health hazards based on false-negative results. In order to achieve compatibility in data, it is recommended that laboratories involved in the analysis of mercury participate in a frequent split-sample analysis program.

Mercury Analysis in Environmental Samples

1.0 SCOPE

1.1 The Aquatic Biology Group at the Alberta Environmental Centre has identified, through client departments, the need for determining the mercury levels in environmental samples such as fish, sediment, water and suspended solids, collected in the Province of Alberta. The available procedures (Magos, 1971; Uthe et al, 1970; Report by the Environmental Contaminants Program Steering Committee of Fisheries and Environment Canada, 1977; Ottawa River Project Report, National Research Council of Canada, 1977) have been reviewed in terms of reference and acceptability and the best suited method for each matrix has been chosen. During method development, due attention was devoted to the quality control measures such as precision, accuracy, spikes and percent recovery. Close co-ordination was developed among those involved (i) in sampling for valid sampling techniques and preserving the integrity of samples prior to analysis, (ii) in performing analyses for routine intra-laboratory quality control program and (iii) in interpreting the data for participation in inter-laboratory quality control studies when available and analysis of standard reference materials. This will assure the quality of the mercury data generated on an integrated basis.

1.2 The available data from federal and provincial agencies (Mercury in the Canadian Environment, Environment Canada, 1979) on fish in Alberta is spread over a period of years during which the analytical methodology was continuously being refined to assure data quality and a lower limit of detection. Hence it is very difficult to place an estimate of quality on existing data on a relative basis. More importantly, none of the data reported in the Province of Alberta included any estimate of the fraction of the most toxic form of mercury, namely, methylmercury. No data exist on the mercury levels in sediments, an important compartment in the mercury flux in the environment. Hence the Aquatic Biology Group developed methods for the analysis of inorganic, methyl and total mercury in environmental samples.

2.0 CHEMISTRY

2.1 Mercury (CH_3Hg^+ and Hg^{2+}) is released from the binding sites in the matrix into solution by a combination of (50%) H_2SO_4, Cl^- and cysteine (for animal tissue), (50%) H_2SO_4, $KMnO_4$, $K_2S_2O_8$, dextrose, cysteine and Cl^- (for sediments) and conc. H_2SO_4, conc. HNO_3, $KMnO_4$, $K_2S_2O_8$, Cl^- - NH_2OH (natural water and effluent), after homogenizing the matrix under analysis.

2.2 Differential Reduction Method

This method is applicable for the determination of inorganic and total mercury in soft dispersible animal tissues only. The sample is digested with 50% H_2SO_4, Cl^- and cysteine and the released Hg- and CH_3Hg - cysteine complexes are reduced with different reducing agents $SnCl_2/NH_2OH$ and $SnCl_2/Cd^{2+}$ to determine the inorganic and total mercury respectively.

2.2.1 Inorganic Mercury Analysis:

The digested solution is treated with a strongly alkaline $SnCl_2/NH_2OH$ solution reducing inorganic mercury (Hg^{2+}) to elemental mercury (Hg^0) and partitioning it into the headspace. The Hg^0 produced is swept by pure N_2 gas into an absorption cell of the Mercury Monitor. CH_3Hg-cysteine complex is not reduced in this step.

2.2.2 Total Mercury Analysis:

Another subsample is treated with a strongly alkaline $SnCl_2/Cd^{2+}$ solution which reduces both Hg^{2+}-cysteine and CH_3Hg-cysteine complexes to Hg^0 which is analyzed by the Mercury Monitor to give the total mercury in the sample.

2.2.3 Methyl Mercury Analysis:

Methylmercury is calculated by difference. The result of 2.2.1 is subtracted from that of 2.2.2 to give the methylmercury content in the sample.

2.2.4 Equations

2.2.4.1 Reduction of inorganic mercury (Hg^{2+}).

$$Hg^{2+} \longrightarrow Hg^0\uparrow + Sn^{4+}$$

$$\text{Hg-cysteine} \xrightarrow[SnCl_2/NH_2OH]{OH^-(\text{excess}),\ Cl^-} Hg^0\uparrow + Sn^{4+}$$

$$CH_3\text{-Hg-cysteine} \xrightarrow[SnCl_2/NH_2OH]{OH^-(\text{excess}),\ Cl^-} \text{No reduction}$$

2.2.4.2 Reduction of total mercury (inorganic and methylmercury, CH_3Hg).

$$\text{Hg-cysteine} \xrightarrow[SnCl_2/Cd^{2+}]{OH^-(excess),\ Cl^-} Hg^0\downarrow + Sn^{4+} + \text{Cd-cysteine}$$

$$CH_3\text{-Hg-cysteine} \xrightarrow[SnCl_2/Cd^{2+}]{OH^-(excess),\ Cl^-} Hg^0\downarrow + Sn^{4+} + \text{Cd-cysteine}$$

2.3 Differential Digestion Method

This method is applicable for the determination of mercury in a wide variety of animal tissues, such as whole or dressed fish, muscle and organs of fish and birds and blood and urine of birds. Aliquots of the sample are digested separately with (i) cysteine mixture and (ii) strong acid mixture and oxidant ($KMnO_4$) at moderate temperatures to yield Hg (and CH_3Hg-) cysteine complexes and Hg^{2+} ions respectively. The two differently digested samples are then reduced with $SnCl_2/NH_2OH$ solution to determine the inorganic Hg in (i) and total mercury in (ii) respectively.

2.3.1 Inorganic Mercury Analysis:

The cysteine-digested sample is reduced with a strongly alkaline $SnCl_2/NH_2OH$ as described in 2.2.1.

Equations:

As described in 2.2.4.1

2.3.2 Total Mercury Analysis:

The sample digested with a mixture of strong acids and oxidant ($KMnO_4$) contains all the mercury (both inorganic and methyl mercury) in the Hg^{2+} form. The sample is reduced with strongly alkaline $SnCl_2/NH_2OH$ to determine the total mercury content.

2.3.3 Methyl Mercury Analysis:

The difference in the values for a given sample for inorganic mercury (2.3.1) and total mercury (2.3.2) gives the methyl mercury content of the sample.

2.3.4 Equations

Oxidation of CH_3Hg in the sample:

$$CH_3\text{-}Hg \text{ (and R-Hg)} \xrightarrow[H_2SO_4 + HNO_3 + KMnO_4]{-e} Hg^{2+}$$

Reduction of Hg^{2+}:

$$Hg^{2+} \xrightarrow[SnCl_2/NH_2OH]{+2e} Hg^0\downarrow + Sn^{4+}$$

3.0 APPARATUS

3.1 LDC (Laboratory Data Control, U.S.A.) Mercury Monitor (Model 1235) equipped with mercury hollow cathode lamp emitting monochromatic light at 253.7 nm.

3.2 Chart recorder.

3.3 Specially designed and constructed pyrex glass mercury reduction - bubbler assembly (Figure 1).

3.4 Glass absorption cell, 30 cm long and 0.75 cm diameter with quartz windows at both ends.

3.5 Heating tape with a variable voltage regulator.

3.6 Pure N_2 gas source (standard cylinder) with two-stage exit regulators for constant gas delivery at low flow rate.

3.7 Constant temperature shaker bath with gable cover, platform and clips to hold 20 or more glass digestion tubes, total capacity 24-35 litres of water.

3.8 Specially constructed pyrex glass digestor-analysis tubes (minimum of 80 tubes is necessary for routine analysis); 150 cm height and 2.5 cm diameter with tapered ground glass 24/40 joint and a pair of side hooks (Figure 2).

3.9 Disposable sterile 8 oz polypropylene/polyethylene cups.

3.10 Millimatic reagent dispensers, five 1 litre dispensers with major and vernier scales.

3.11 Volumetric flasks of 250, 500 ml and a litre capacity.

3.12 Pipetman and disposable tips for microvolume dispensing.

3.13 Magnetic stirrer.

3.14 Stop watch.

3.15 All-glass tissue grinding tubes with stainless steel shaft on the plunger.

3.16 1/2 inch reversible variable speed electrical hand drill.

3.17 Homogenizer; sorvall omni-mixer, Polytron PT10 or standard electrical blender with special mini-blending jars.

3.18 40-tube plexi-glass racks.

3.19 Specially constructed mercury-reduction apparatus (an alternative to 3.8) (Figure 3).

3.20 Laboratory jack.

4.0 REAGENTS

4.1 Tissue digestion acid mixture: Add 100 ml of HNO_3 (sp.gr. 1.42) to 400 ml of H_2SO_4 (sp.gr. 1.84) with care under ice. Mix and cool.

4.2 Sediment digestion acid mixture: Mix carefully 200 ml of conc. H_2SO_4 (sp.gr. 1.84) with 100 ml of conc. HNO_3 (sp. gr. 1.42) under ice.

4.3 Sulphuric acid (0.1N): Dilute 2.8 ml Baker Analyzed or equivalent grade conc. H_2SO_4 (sp.gr. 1.84) to 1L with distilled-deionized water (d.d.w).

4.4 Sulphuric acid (16N): Dilute 444 ml of Baker Analyzed or equivalent grade conc. H_2SO_4 (sp.gr. 1.84) to 1L with d.d.w.

4.5 Sulphuric acid (1N): Dilute 28 ml of Baker Analyzed or equivalent grade conc. H_2SO_4 (sp. gr. 1.84) to 1L of d.d.w.

4.6 Sodium chloride solution (20% w/v): Dissolve 100 g of Baker Analyzed or equivalent grade NaCl in 500 ml d.d.w. and store in a millimatic dispenser.

4.7 Hydrochloric acid solution (5N): Dilute approximately 41 ml of Baker Analyzed or equivalent grade conc. HCl to 1L with d.d.w.

4.8 Cysteine solution (1% w/v): Dissolve 10 g of high purity grade L(+)-cysteine hydrochloride (H_2O) in 25 ml of Baker Analyzed or equivalent grade 5N HCl and make up to 1L in d.d.w. Store in a millimatic dispenser.

4.9 Stannous Chloride solution (10% w/v): Dissolve 10 g of MCB Reagent grade grade $SnCl_2$, $2H_2O$ in d.d.w. and make up to 100 ml.

4.10 Sodium hydroxide solution (45% w/v): Dissolve 900 g of Baker or equivalent grade NaOH in approximately 1800 ml of d.d.w. and make up to 2L. Add 10 ml of 10% $SnCl_2$ solution, bubble N_2 vigorously through the solution for an hour to eliminate mercury impurity and then store in a Teflon coated millimatic dispenser or in a polypropylene container.

4.11 Hydroxylamine sulphate solution (40% w/v): Dissolve 40 g of $(NH_2OH)_2.H_2SO_4$ in d.d.w. and make up to 100 ml.

4.12 Stannous/hydroxylamine reducing solution: Dissolve 5 g of MCB $SnCl_2$, $2H_2O$ and 7.1 ml of 40% hydroxylamine sulphate (4.11) in 100 ml of 1N H_2SO_4 (4.5) with heating. Aerate for 10 minutes to remove any mercury present. Store in a polypropylene container. (Discard after 1 week).

4.13 Stannous-cadmium reducing solution: Dissolve 50 g of MCB $SnCl_2.2H_2O$ and 10 g of anhydrous $CdCl_2$ in approximatley 80 ml

of 1N H_2SO_4 with heating and dilute to 100 ml with 1N H_2SO_4. Aerate for 10 minutes. (Discard after 1 week).

4.14 Potassium permanganate solution (6% w/v): Dissolve 60 g of Reagent grade $KMnO_4$ in 1L d.d.w. If mercury contamination is suspected as seen from blank measurement, add 25 ml of 0.4M $FeSO_4$ in 0.75N H_2SO_4, followed by slow addition of 10 ml of 45% NaOH solution. Let stand overnight, centrifuge to remove flocculated ferric hydroxide. Decant the supernatant into a millimatic dispenser.

4.15 Hydrogen peroxide: H_2O_2, 30% is commercially available in pure grade.

4.16 Potassium persulphate solution (5% w/v): Dissolve 5 g of Baker or equivalent grade $K_2S_2O_8$ in 100 ml of d.d.w. (Discard after 1 week).

4.17 Sodium chloride-hydroxylamine sulphate solution (12% w/v): Dissolve 12 g of high purity NaCl and 12 g $(NH_2OH)_2.H_2SO_4$ in d.d.w. and dilute to 100 ml. Hydroxylamine chloride may be used in place of hydroxylamine sulphate. (Discard after 1 week).

4.18 Hydroxylamine sulphate - Sodium chloride solution (6% w/v): Dissolve 30 g of $(NH_2OH)_2\ H_2SO_4$ and 30 g of NaCl in 500 ml of d.d.w. (Discard after 2 days).

4.19 Dextrose solution (20% w/v): Dissolve high purity L-dextrose in 100 ml d.d.w. (Discard after 1 week).

4.19.1 Stock inorganic mercury standard solution (100 mg/l): weigh exactly 0.1354 g of reagent grade $HgCl_2$ and dilute with 1N H_2SO_4(4.5) to a liter in a volumetric flask.

4.19.2 Intermediate inorganic mercury standard solution (200 µg/l): dilute 1 ml of stock solution (4.19.1) to 500 ml with 1N H_2SO_4(4.5). Discard after 1 week.

4.19.3 Working inorganic mercury standard solutions (0.1-1.0 µg/l): prepare daily before analysis the following working standards in 500 ml of 1N H_2SO_4(4.5):

ml of intermediate standard (4.19.2)/500 ml	Conc. Hg, ng/ml
0.250	0.10
0.625	0.25
1.250	0.50
1.875	0.75
2.500	1.00

4.19.4 If needed, prepare working standards at higher range by proper dilution of the intermediate solution (4.19.2).

4.20.1 Stock methylmercury standard solution (100 mg/l): weigh exactly 0.1252 g. of reagent grade methyl mercuric chloride, dissolve in 5 ml of acetone (if needed) dilute to 1000 ml with 1N H_2SO_4(4.5).

4.20.2 Intermediate methylmercury standard solution (200 µg/l): dilute 1 ml of stock solution (4.20.1) to 500 ml with 1N H_2SO_4(4.5). Discard after 1 week.

4.20.3 Working methylmercury standard solution (0.1-1.0 µg/l):

ml of intermediate standard (4.20.2)/500 ml	Conc. CH_3Hg,ng/ml
0.250	0.10
0.625	0.25
1.250	0.50
1.875	0.75
2.500	1.00

4.20.4 If needed, prepare higher range working standards by proper dilution of the intermediate solution (4.20.2).

5.0 PROCEDURE - FISH TISSUE:

5.1 Sampling procedure:

5.1.1 Record the field data such as date, location, species, length, weight and any other relevant information needed in interpreting the results.

5.1.2 Use plastic containers with tight-fitting lids or plastic bags with good seal and thick walls to prevent moisture and other loss due to diffusion. Keep the head space to a minimum.

5.1.3 Take care not to contaminate the containers in the field.

5.1.4 Obtain a representative sample; avoid pooling of samples.

5.1.5 Select tissues that are least susceptible to the overall state of the fish. Gonadal tissues vary within the fish's reproductive cycle and thus are poor for monitoring purposes. Muscle and liver are better, though they might suffer from some variation as energy sources for the animal during lean periods.

5.2 Sample preservation:

5.2.1 Freeze the samples using dry ice if necessary and transport to the laboratory as soon as possible.

5.2.2 Store the fresh samples in the refrigerator at 2°C for 2-3 days prior to analysis.

5.2.3 If delay in analysis longer than 3 days is anticipated, deep freeze the samples at -20°C until analysis.

5.3 Sample preparation:

5.3.1 Weigh directly small pieces of tissues such as muscle, liver etc. into the tissue grinder or blender with half the weight of 0.1N H_2SO_4 (i.e. 20 g muscle, 10 g 0.1N H_2SO_4).

5.3.2 Blend for 3 minutes or until a smooth paste is obtained.

5.3.3 Aliquot accurately around 1 g of the wet homogenate into four digestion tubes, stopper them and store them briefly in a refrigerator. (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

5.3.4 Aliquot accurately around 1 g of each tissue into a petri dish; leave the dish with lid loosely closed in a forced-air oven for 24 h at 55°C.

5.3.5 Weigh the dry tissue and ensure reproducible dry weight is obtained. Calculate the wet:dry weight ratio for each fish tissue.

5.3.6 If a delay (more than one day) in analysis is anticipated, store in a freezer (-20°C) the aliquots in 8 oz disposable sterile polypropylene/polyethylene cups sealed with lids.

5.3.7 The aliquots in the polyethylene cups are analyzed in total for their mercury content.

5.4 Sample digestion: for analysis by Differential Reduction Method.

5.4.1 To each of the four subsamples in the digestion tubes (5.3.3) add in the following order: 1 ml of 20% NaCl solution (4.6), 1 ml of 1% cysteine solution (4.8) and 10 ml of 16N H_2SO_4 (4.4). (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

5.4.2 Stopper the digestor tubes tightly and fit them in the tube

rack and leave them shaking in shaker water bath at 55°- 60°C for one hour.

5.4.3 Ensure good mixing in the tubes by adjusting the shaking speed.

5.4.4 If samples are not completely dispersed, leave them shaking in the bath for an additional 0.5 hr.

5.4.5 Remove the tubes and cool to room temperature.

5.4.6 Rinse down the stopper and the side of the tube with minimum d.d.w. and make up to constant volume, either 15 ml or 20 ml.

5.4.7 Proceed to analysis within 24 hr.

5.5 For Analysis by Differential Digestion Method:

5.5.1 To two of the four subsamples in the digestion tubes (5.3.3) add in the following order; 1 ml of 20% NaCl solution (4.6), 1 ml of 1% cysteine solution (4.8) and 10 ml of 16N H_2SO_4 (4.4). Use disposable 8 oz. plastic cups if the alternate reduction apparatus (3.19) is used.

5.5.2 To the remaining two subsamples in the digestion tubes

(5.3.3), add 5 ml of the digestion acids mixture (4.1); ensure that the sample is totally immersed in the acid.

5.5.3 Stopper the digestor tubes tightly, fit them in the tube rack and leave them shaking in the water bath at 55 - 60°C for 2 hours or until a clear solution is obtained.

5.5.4 Remove the tubes and cool them to the room temperature.

5.5.5 Add slowly 15 ml of 6% $KMnO_4$ solution (4.14) and let stand for at least 30 minutes. If the purple color disappears at this time, add more $KMnO_4$ solution till the color persists for 30 minutes.

5.5.6 Add 30% H_2O_2 solution (4.15) with caution while stirring until the precipitate just dissolves.

5.5.7 Make up the solution to a known volume and mix thoroughly.

5.5.8 The samples are ready for analysis.

5.6 Analysis:

5.6.1 Setting up the mercury monitor: interconnect the optical and control units with the cable provided. (Refer to instruction manual).

5.6.2 Connect the terminals of 0-10 mV recorder to the control unit. (Refer to instruction manual).

5.6.3 Connect the inlet of the reference cell to the N_2 source using 1/16 or 1/8 inch air tubing; the outlet of the reference cell to the gas bubbler inlet; the outlet from the bubbler to the inlet of sample cell; and bubble the outgoing gas through $KMnO_4$ solution in a 1 L Ehrlenmyer flask which collects all the mercury vapour.

5.6.4 Ensure that the recorder is properly grounded.

5.6.5 Turn the power switch on. The circuit monitor meter should immediately go to a high value (or off-scale). After a few seconds, the source lamp will light and the meter should return to 0.04 to 0.6. If meter reading is over 0.6, refer to mercury monitor manual P-14 for trouble-shooting.

5.6.6 Adjust the voltage regulator for the cell heating tape to give a temperature of approximately 60°C.

5.6.7 Allow the meter to warm up for at least 1 hr; preferably leave it on unless a long delay in use (more than 2 days) is anticipated.

5.6.8 Position the wave length selector switch to 254 nm.

5.6.9 Zero adjustment of the system: place the range switch on the control unit to "zero" or "S".

5.6.10 With the recorder zero control, position the recorder pen at 50 % deflection and allow the system to stabilize.

5.6.11 Set the mercury monitor zero control at mid-position, 5.0 on the dial.

5.6.12 Bring the attenuator screws, located behind the photo detector head on both sides of the cell assembly, to the outer most position. Set the monitor range switch to "0.16" and adjust the attenuator screws until recorder shows 50 % deflection.

5.6.13 Place the monitor range switch to "zero" and with recorder zero control, set the recorder pen to indicate zero on the chart paper.

5.6.14 Switch the range switch to "0.16" and readjust the recorder pen to zero on the chart paper.

5.6.15 After stability is achieved on "0.16" range, setting may be changed during operation to permit greater sensitivity for small peaks or to bring down off-scale large peaks. Ensure a steady base line at each range setting.

5.6.16 The system is ready for analysis.

5.6.17 Inorganic Mercury Analysis (same for both differential reduction method and differential digestion method)

Calibration

(i) pipette exactly 15 ml of inorganic mercury standard solution (4.19.3) into digestor-analysis tubes. (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

(ii) add to each tube 1 ml of 20% NaCl solution (4.6), 1 ml of 1% cysteine solution (4.8) and 3 ml of 16N H_2SO_4(4.4).

(iii) place the tube in position with the bubbler and flush it with N_2 gas to purge any interfering vapors.

(iv) make sure not to leave any solution in the bubbler tube.

(v) add 1 ml of $SnCl_2/NH_2OH$ solution (4.12) and 2 ml of 45% NaOH solution (4.10) in quick succession.

(vi) flush the mercury vapour produced with N_2 gas after 30 seconds (use a stop watch) into the monitor and record the peak absorbance.

(vii) when the absorbance begins to decline, turn the 4-way value to bypass position and rinse off the bubbler tube.

(viii) analyze next sample as above.

(ix) analyze blanks (solution containing all reagents except mercury) as above.

(x) stopper the tubes at each step.

(xi) Refer to Figure 4 for the flow diagram in a typical mercury analysis.

5.6.18 Analysis of Samples

Analyze the samples following steps from 5.6.17, (ii) to (x).

5.6.19 Total Mercury Analysis: (differential reduction method).

Calibration

(i) pipette exactly 15 ml of total mercury standard 1:1 mixture of inorganic and methylmercury standards, (4.19.3 and 4.20.3); 15 ml of inorganic and 15 ml of methylmercury standard solutions into digestor-analysis tubes and stopper them. (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

(ii) digest the standards, following steps 5.4.1 to 5.4.7.

(iii) go through steps 5.6.17 (iii) and (iv) as described.

(iv) add 2 ml of $SnCl_2$/Cd solution (4.13) and 2 ml of 45% NaOH solution (4.10) in quick succession.

(v) go through steps 5.6.17 (vi) to (x) as described.

5.6.20 Sample analysis: analyze the digested samples (5.4.7) for inorganic and total mercury content by following steps 5.6.17(ii) to (x) and 5.6.19(ii) to (iv) respectively.

5.6.21 Total Mercury Analysis (differential digestion method)

Calibration

(i) Pipette exactly 15 ml of the total mercury standards as described in 5.6.19, (i).

(ii) Follow the steps from 5.5.2 to 5.5.8.

(iii) Analyze for the total inorganic mercury following the steps from 5.6.17 (v) to (x).

5.6.22 Analysis of Samples

Analyze the samples as described in 5.6.21, (iii) for the total mercury.

5.7 Calculations:

5.7.1 After correcting for blank readings, if any, plot the peak heights (P.H.) versus amount of Hg.

5.7.2 Prepare separate calibration curves for inorganic and total mercury.

5.7.3 Compare the sample peak heights with the calibration curves and determine the inorganic and total mercury concentrations.

5.7.4 The difference between the average total mercury and average inorganic mercury is methylmercury.

5.7.5 $$\text{Hg content in tissue} = \frac{\text{Amount of Hg(inorganic/methyl)}(\mu g)}{\text{weight of subsample (g)}}$$

6.0 PROCEDURE - SEDIMENT:

6.1 Sampling procedure:

6.1.1 Collect samples using an appropriate sampling device depending on the requirements; Ekman dredge, grab sampler, core sampler and air-lift sampler (constructed by the Aquatic Biology Group) are the commonly used samplers.

6.1.2 Drain most of the water from the samples immediately after collection.

6.1.3 Obtain a representative field sample.

6.1.4 Store them in polyethylene/polypropylene containers with minimum head space.

6.1.5 Seal the containers tightly and transport them as quickly as possible to the laboratory.

6.2 Sample preservation:

6.2.1 No chemical preservative is required.

6.2.2 Store the samples at -20°C in plastic containers. Use dry ice for field preservation.

6.3 Sample preparation:

6.3.1 Determine the moisture content in the sample; weigh a representative portion of the sediment sample in a dry tared beaker; dry the sediment sample gently in a forced air oven at about 45°C; determine the dry weight of the sample.

6.3.2 Weigh a homogenized wet sample accurately 1 gm (or less for organic - rich sediment) into four digestor-analysis tubes. (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

6.3.3 If a delay in analysis is anticipated, stopper tubes and store in a refrigerator (not more than one day).

6.3.4 Nearly all of the mercury in sediment is known to be inorganic mercury. Hence for all trend-monitoring purposes it is enough to determine the total mercury content.

6.4 Sample digestion:

6.4.1 <u>For inorganic mercury analysis</u>.
Add 10 ml of 16N H_2SO_4(4.4) to two tubes (6.3.2) and set the tubes in a rack.

6.4.2 Place the rack in a constant temperature shaker bath at

55-60°C and leave the tubes shaking for 1 hour.

6.4.3 Take the tubes out and let them cool to room temperature.

6.4.4 Add 10 ml of 6% $KMnO_4$(4.14) and let the solution stand at room temperature for 24 hours.

6.4.5 Two hours prior to analysis, add 1 ml of 5% $K_2S_2O_8$(4.16), cap the tubes and let stand for 1.5 hours.

6.4.6 Add 3 ml of 20% dextrose solution (4.19) with swirling until the purple color disappears. If the purple color disappears before the addition of dextrose, repeat the addition of $KMnO_4$ and $K_2S_2O_8$ solution.

6.4.7 Add 1 ml of 1% cysteine solution (4.8), swirl the solution occasionally until all of the MnO_2 is reduced (variable up to several minutes).

6.4.8 Add an additional 1 ml of 1% cysteine solution (4.8) and 1 ml of 20% NaCl (4.6).

6.4.9 Make up to a constant volume; if the volume is too large for analysis, in the tube, pipette an aliquot into a clean tube.

6.4.10 The solution is ready for analysis.

6.5 For total mercury analysis.

6.5.1 Rinse the samples in the plastic cups (6.3.2) down with minimum amount of (not exceeding 10 ml) of d.d.w.

6.5.2 Add carefully 15 ml of digestion acid mixture (2.1 H_2SO_4:HNO_3) (4.2) under ice.

6.5.3 Cool well and then add 2 ml of 12N HCl.

6.5.4 Place the cups in a shaker water bath at 60° C and digest with shaking for 2 h.

6.5.5 Remove the cups from the bath and cool them for 30 min.

6.5.6 Add very slowly 15 ml of $KMnO_4$ solution (6% w/v) (4.14) with caution, while continuously stirring in a fume hood.

6.5.7 Let stand for 30 min. If purple colour is not persistent at this stage, repeat the addition of $KMnO_4$.

6.5.8 Add 5 ml of Potassium persulphate solution (5% w/v) (4.16) with gentle stirring.

6.5.9 Let stand overnight.

6.5.10 Add 10 ml of hydroxylamine sulphate-sodium chloride solution (6% w/v) (4.18). Mix thoroughly until the solution is clear.

6.5.11 Make up the solution to a known volume.

6.5.12 Analyze the solution the same day.

6.6 Analysis

6.6.1 Analyze the digested solutions (6.4.10 and 6.5.11) following steps 5.6.1 to 5.6.18.

6.7 Calculations

6.7.1 Follow steps 5.7.1 to 5.7.5.

7.0 PROCEDURE - WATER SAMPLE:

7.1 Sampling procedure:

7.1.1 Pre-rinse the polypropylene containers by soaking them for 24 hours in 50% HNO_3 and rinsing five times d.d.w.

7.1.2 Collect samples (one litre each) in polypropylene containers with the preservative in (7.2.1)

7.1.3 Avoid any head space in the container.

7.1.4 Obtain a representative sample; take special care in sampling turbid waters.

7.2 Sample preservation:

7.2.1 For a 100-ml aliquot, add 1 ml H_2SO_4 (sp. gr. 1.84) and 1 ml of 5% (w/v) Potassium dichromate solution as perservative.

7.2.2 Store the samples at 4°C.

7.3 Sample preparation.

7.3.1 Pipette exactly 15 ml of the preserved water sample into digestor-analysis tubes. If high particulate matter is present, homogenize the sample prior to taking an aliquot. (Use disposable 8 oz plastic cups (3.9) instead of tubes, if the alternate reduction apparatus (3.19) is used).

7.3.2 For particulate mercury analysis, filter the sample in an acid-washed all-glass apparatus and transfer a known volume of aliquot; re-suspend the known weight of particulate in D.D. water for direct mercury analysis.

7.3.3 Almost all of the detectable mercury in water is inorganic mercury. Hence the water samples are analyzed for the total mercury content only.

7.4 Sample digestion:

7.4.1 Add 0.5 ml conc. H_2SO_4 and 0.25 ml conc. HNO_3 to each tube.

7.4.2 Add 0.1 ml of 6% $KMnO_4$(4.14) to each tube and allow to stand for a minimum of 15 minutes. If purple color disappears at this time, add more $KMnO_4$ till color persists for 15 minutes.

7.4.3 Add 0.2 ml of $K_2S_2O_8$(4.16) to each tube, mix well and place the tubes in a rack.

7.4.4 Leave the tubes shaking in a water bath at 55-60°C for 2 hours.

7.4.5 Remove the samples from the bath and allow to cool to room temperature.

7.4.6 Add 0.2 ml of NaCl-NH_2OH sulphate solution (12% w.v) (4.17) to clear the solution.

7.4.7 The sample is ready for analysis.

7.5 Analysis

7.5.1 Follow steps, 5.6.1 to 5.6.18.

7.6 Calculations

7.6.1 Follow steps, 5.7.1 to 5.7.5.

8.0 QUALITY CONTROL PROCEDURES

8.1 Method development

8.1.1 Calibration: Ensure stability of instrumental response to a 'known' concentration of the analyte.

8.1.2 Complete calibration before every run commences and ensure linearity.

8.1.3 Run quality control solutions to monitor in-run and between-run stability of the calibration.

8.1.4 Trouble shoot in case of
(i) significant change in instrumental response
(ii) non-linear calibration and/or
(ii) evidence of bias.

8.1.5 Identify the sources of blank error and make appropriate correction; avoid over-correction unless a change in the blank is confirmed.

8.1.6 Recovery from different matrices: spike the matrix of analysis:
(i) synthetic standard solution
(ii) natural and industrial waters
(iii) biological tissue and

(iv) sediment

with a known amount of the analyte.

8.1.7 Apply the total analytical procedure to each matrix and determine the percent recovery of the spike.

8.1.8 Failure to recover the spike may be due to:

(i) loss during extraction

(ii) inefficiency of the sample digestion method

(iii) breakdown of the form of the analyte and the resultant insensitivity of the analytical methodology to detect and/or

(iv) matrix effects.

8.1.9 Confirm that the recovery factor is precise and consistent with previous analytical runs:

(i) based on more than one or two analyses and

(ii) free from analytical imprecision.

8.1.10 Precision: Determine precision of the method for every matrix by analyzing identical duplicates; split the sample, analyze in the same run and analyze at different runs.

8.1.11 In case of failure to reproduce results for identical duplicates, check on sample non-homogeneity, lack of matching analytical conditions, electronic noise and out-of-control analytical system and bring the random errors under control to assure precision.

8.1.12 Accuracy: use in-house reference materials and standards as control to demonstrate the accuracy of measurement.

8.1.13 Determine the coefficient of variation at both low range and high range of analysis.

8.1.14 Evaluate performance against accepted Standard Reference Materials (SRM's). Accuracy cannot be demonstrated until routine documentation of continuing precision, and recovery is available.

8.1.15 Specificity: Demonstrate the analytical capability of the method to distinguish the analyte from other species which may respond similarly under test conditions, through checks on

(i) documentation of nature of samples causing recovery problems

(ii) testing the suspected interferants and blanking them by appropriate chemical methods (for example, interference by chlorine at 253.7 nm) and

(iii) alternative analytical procedures, if available.

8.2 Intra-laboratory Routine Quality Control Procedure:

8.2.1 Since the Mercury Monitor is extremely sensitive to Hg vapours the analyst should ensure that the laboratory is free from possible sources of contamination such as a polarograph or broken thermometer.

8.2.2 Run a d.d.w. blank to set 100% transmission and a reagent blank to adjust the baseline.

8.2.3 Check the calibration curve using standards.

8.2.4 Preparation of A,B control samples: prepare two known Quality Control (Q.C.) solutions independent of calibration standards (or natural samples) responding to 10% (A) and 60% (B) of full scale in a single analytical linear range.

8.2.5 Prepare a new pair of Q.C. Solutions and analyze for at least 3 days before the old set is exhausted.

8.2.6 Prepare in sufficient quantity to last for at least 20 runs ($\sim$1 month).

8.2.7 Ensure the stability of Q.C. Solutions samples through proper storage and handling.

8.2.8 Take extreme care not to contaminate Q.C. Solutions.

8.2.9 Analyze the Quality Control Solutions (A,B) per run or batch of samples, immediately after the calibration for that run or batch. This is to check on the control on within-run and between-run calibration.

8.2.10 Plot the difference (D=A-B) and sum (T=A+B) of the A and B solutions results on a control chart of concentration versus time with respect to their established mean values, $\bar{D}$ and $\bar{T}$ respectively. Maintain a sequential plot of the values over time. (Figure 5).

8.2.11 Calculate the standard deviation of the data and set the warning and control limits within which the points (A-B) and (A+B) are expected to fall (Figure 5).

8.2.12 s_D = standard deviation of difference (A-B)

$$= \sqrt{\frac{\Sigma(D^2) - (\Sigma D)^2/n}{(n - 1)}}$$

given D = (A-B)

and n = number of A,B data pairs

8.2.13 Warning limits: $(A-B) \pm 2S_D$ and $(A+B) \pm 2S_D$

(Warning limit: Less than 5% of data will exceed the average value)

Control limits: $(A-B) \pm 3S_D$ and $(A+B) \pm 3S_D$

(Control limit: Less than 0.2% of data will exceed the average value).

8.2.14 Evaluation of Data

(i) When results of (A-B) lie within the control limits imposed on $\bar{D}$ (Figure 5), the slope is in control. If not, significant degradation in the analytical process is suspected. Attention must be paid to individual values of A and B and other random errors contributing to out-of-control slope.

(ii) If the slope is in control, an outlier in (A+B) (Figure 5) strongly indicates the presence of significant systematic error in the blank or intercept.

(iii) When both (A-B) and (A+B) are within the control limits (Figure 5), the analytical quality is under control.

8.2.15 Analyze a random pair of duplicates in each batch of 10 samples.

8.2.16 Analyze field duplicates every ten samples to detect occasional non-representative samples.

8.2.17 Analyze digested blanks once for each run.

8.2.18 Use double the weight of the sample size in duplicate-subsample to monitor matrix effects.

8.2.19 Run one standard reference material on an occasional basis.

8.2.20 Run a blank, low and high standard (undigested) every 20 samples. A 5% drift is allowed without correction.

8.2.21 Check recovery of spike from fish/sediment matrix once per batch.

8.2.22 Run blanks at the end of each run.

8.2.23 Run A,B quality control samples at the end of each run.

8.2.24 Maintain a log of all quality control data, with notes on unusual changes in performance, eg. excess noise, change in peak shape, poor response with certain quality of chemicals, etc.

8.3 Analytical reproducibility evaluation: Within-run Precision, by analyzing duplicates every ten samples.

8.3.1 Scope: determine the acceptability of precision limits on day-today basis, document the reproducibility of analysis at varying concentration ranges and calculate the within-run standard deviation for the analytical method.

8.3.2 Data handling: record the data in a special form (Figure 6). The vertical axis represents difference between duplicates and each square represents one unit of difference. The factor chosen should ≤ smallest reading interval. Thus for a factor of 0.001 mg L^{-1}, the vertical axis will be labelled 0.000, 0.001, 0.002, 0.003, etc.

8.3.3 The horizontal axis represents concentration divided into 20 parts, covering the operating range in steps of 5%.

8.3.4 The samples falling within the specified concentration range and duplicate difference range fill the same square. A dot represents one sample and a bar five samples. As data become available, they are added on to the appropriate square (Figure 7).

8.3.5 Differences between duplicates are expected to occur. If none is observed, read the next significant figure to read the difference.

8.3.6 Count the number of samples for a given difference (D) for each concentration range (0-20, 20-50 and 50-100% of full scale) and enter the numbers (n) in the corresponding line in the table.

8.3.7 Multiply D^2 (given in the table) by n and enter the results.

8.3.8 Sum the columns to arrive at Σ n and Σ nD^2 for each of the three concentration ranges.

8.3.9 Calculate the scaled variance, $V = (\Sigma\ nD^2/2\Sigma n)$ and enter the data.

8.3.10 Calculate the standard deviation; $S = f\sqrt{v}$.

8.3.11 Sum the number of samples for each column on the top row of the diagram. This gives the sample distribution versus concentration range.

8.3.12 Periodically review the pattern.

8.3.13 Identify and correct excessive deviation which indicates deterioration of routine performance.

8.4 Precision and accuracy of the method evaluated by the Aquatic Biology Laboratory:

8.4.1 Detection Limit:

water: 0.1 ng/ml

fish: 0.2 ng/g

sediment: 0.2 ng/g

8.4.2 Recoveries from Standard Reference Materials, 95-97% (concentration ranges 0.24 to 1.12 µg/g of Hg in SRM's).

8.4.3 Accuracy:

Detection limit	Variation in accuracy
0.1 ng/ml (water)	± 3%
0.2 ng/g (fish & sediment)	± 5%

8.4.4 Precision:

Concentration range	Reproducibility
(i) 0 - 15 ng/g	93%
(ii) 15 - 37.5 ng/g	93%
(iii) 37.5 - 75.0 ng/g	94%

(refer to Fig. 7 for details)

8.5 Inter-laboratory Quality Control Studies:

8.5.1 The Water Quality Branch of National Water Research Institute, CCIW, Burlington, Ontario conducts National Quality Control Programs on water samples. Participate in the program.

8.5.2 The Industry Services Branch, of Fisheries and Environment, Winnipeg, Canada conducts a collaborative study for mercury in fish. Fish homogenates, canned in sets of four at different concentrations are sent out to participants two or three times a year. Participate in the program.

8.5.3 In addition, various U.S. state and federal agencies conduct intercomparison studies. Participation is encouraged.

9.0 GLOSSARY OF STATISTICAL TERMS

Precision:	Reproducibility of the analytical results for replicates on a homogeneous sample. This is the range within which random deviations are expected to fall.
Within-run precision:	Statement of range of deviation in reproducibility under matching conditions in a given analytical run.
Between-run precision:	Statement of range of deviation in reproducibility between runs with fresh calibrations.
Accuracy:	Agreement between the measured value of the analyte and the amount actually present.
Limit of Detection:	Lowest reliably detectable analyte concentration. Usually 3S above the measured average blank.
Detection Limit (95%):	Ensures that when analyte is present, it will be reported absent less than 5% of the time.
Deviation:	Unavoidable random fluctuations in measurements.

Error: Occasional gross deviations, beyond normally expected.

Relative error: Difference between the measured and the actual amounts as a percentage of the actual amount.

Systematic error: Reproducible deviation resulting from improper calibration, blank problems for a given set of analyses.

Standard deviation: Measure of reproducibility of the analytical results. It can be determined in one of two ways:

a) a sample is analyzed n times to obtain results X_1, X_2 ... X_n. The average value $\bar{X}=\Sigma X/n$. The deviations $(X_n-\bar{X})$ are used to calculate the standard deviation:

$$s = \sqrt{\frac{\Sigma(X_i-\bar{X})^2}{n-1}} = \sqrt{\frac{\Sigma X_i^2-(\Sigma X_i)^2/n}{n-1}}$$

b) n samples are analyzed in duplicate; the difference in results $(X_1-X_2)_1$ ... $(X_1-X_2)n$ are used to calculte the standard deviation:

$$s = \sqrt{\frac{\Sigma(X_1-X_2)_n^2}{2n}}$$

Both the estimates are equally valid and can be used to predict the range of finding a result in matching analytical conditions. 68.27% of the observations lie between $\bar{X} \pm 1S$, 95.45% between $\bar{X} \pm 2S$ and 99.70% between $\bar{X} \pm 3S$. Therefore the difference between any two single results will be within $\pm 1.96\sqrt{2}\,S$, 95% of the time, where the $\sqrt{2}$ factor accounts for the variability in both results.

The standard deviation used should be appropriate to the concentration level reported.

Coefficient of variation: Ratio of the standard deviation to the average, expressed as percentage:

$$C.V. = \frac{100S}{\bar{X}}$$

10.0 REFERENCES

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10.2 Mercury: Methods for sampling, preservation and analysis: A Report to the Environmental Contaminants Program Steering Committee of Fisheries and Environment Canada, 1977.

10.3 Ottawa River Project; Distribution and Transport of Pollutants in Flowing Water Ecosystems, Final Report, 1977.

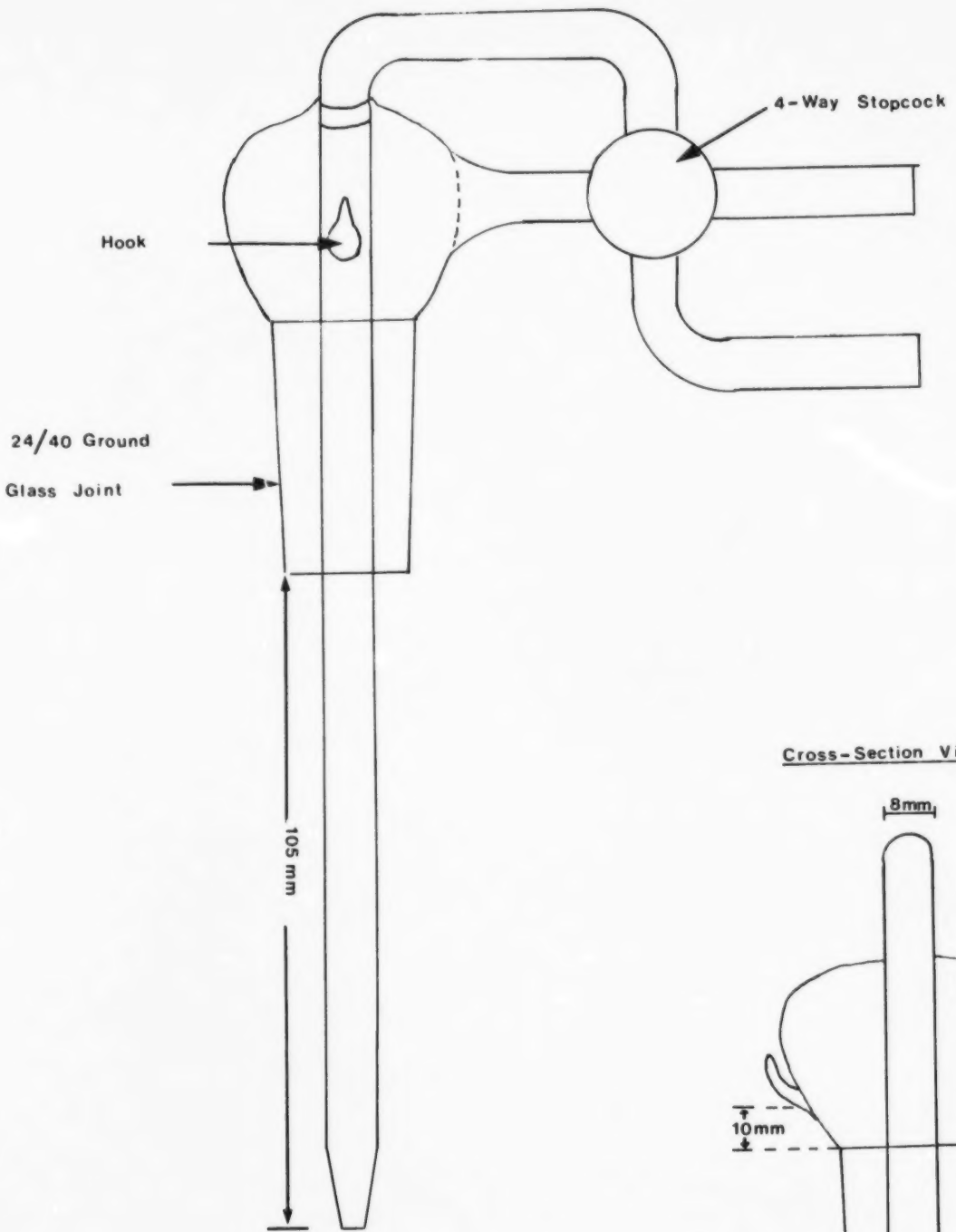
10.4 Quality Control and Data Evaluation Procedures, Ministry of the Environment, Ontario, 1975.

10.5 Sherbin, I.G., Mercury in the Canadian Environment, Environment Canada, EPS Report Series, EPS 3-EC-79-6, 1979.

10.6 UTHE, J.F., ARMSTRONG, F.A.J., and STAINTON, M.P., Mercury Determination in Fish Samples by Wet Digestion and Flameless Atomic Absorption Spectrophotometry. J. Fish. Res. Bd. Canada, 27, 805-811, 1970.

10.7 Youden, W.J., and E.H. Steiner., Statistical Manual of the Association of Official Analytical Chemists, Washington, D.C., 1975.

Figure 1. Pyrex glass bubbler assembly.


4-Way Stopcock
Hook
24/40 Ground
Glass Joint
105 mm

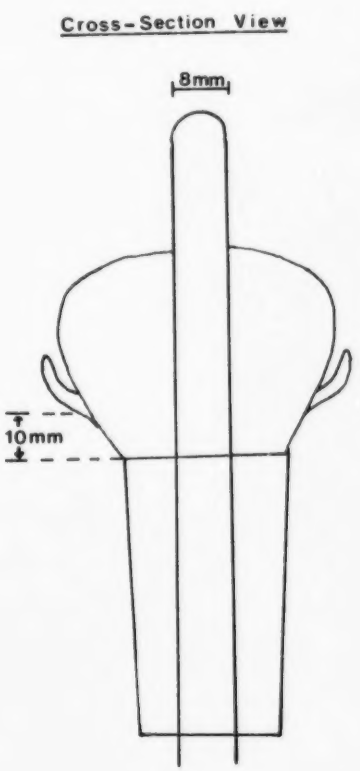

Cross-Section View
8mm
10mm

Figure 2. Digestor-analysis tube.

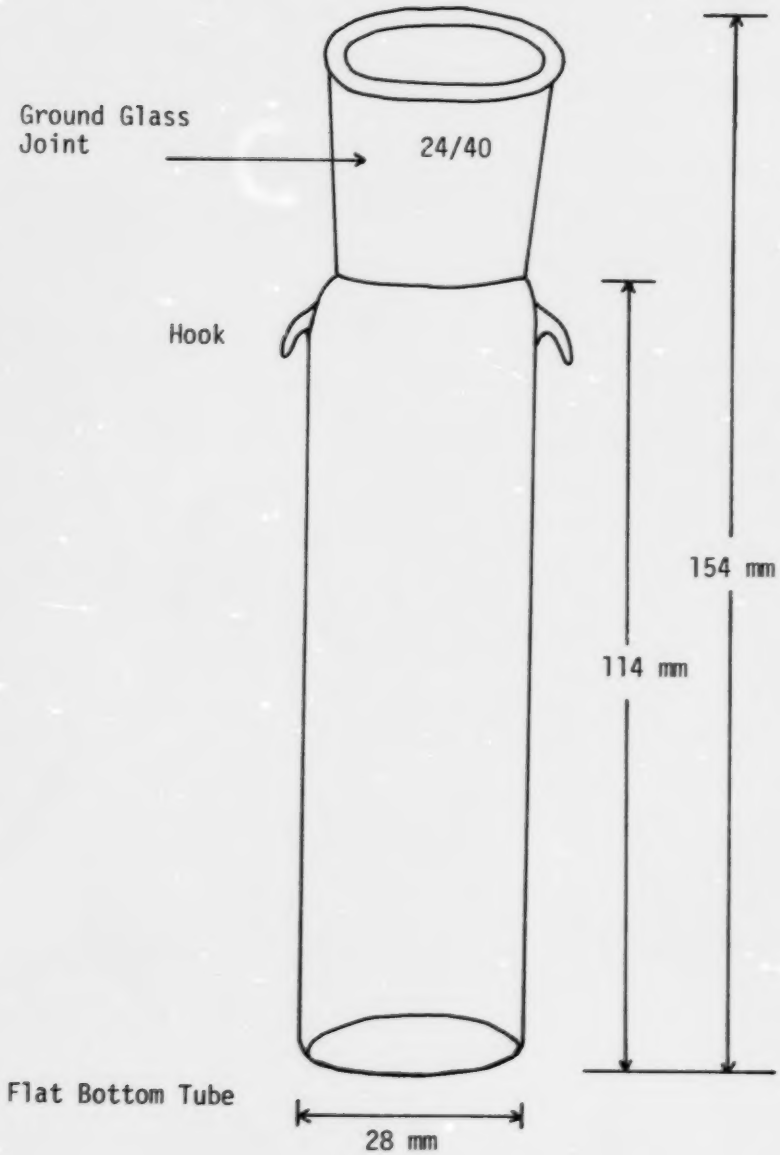
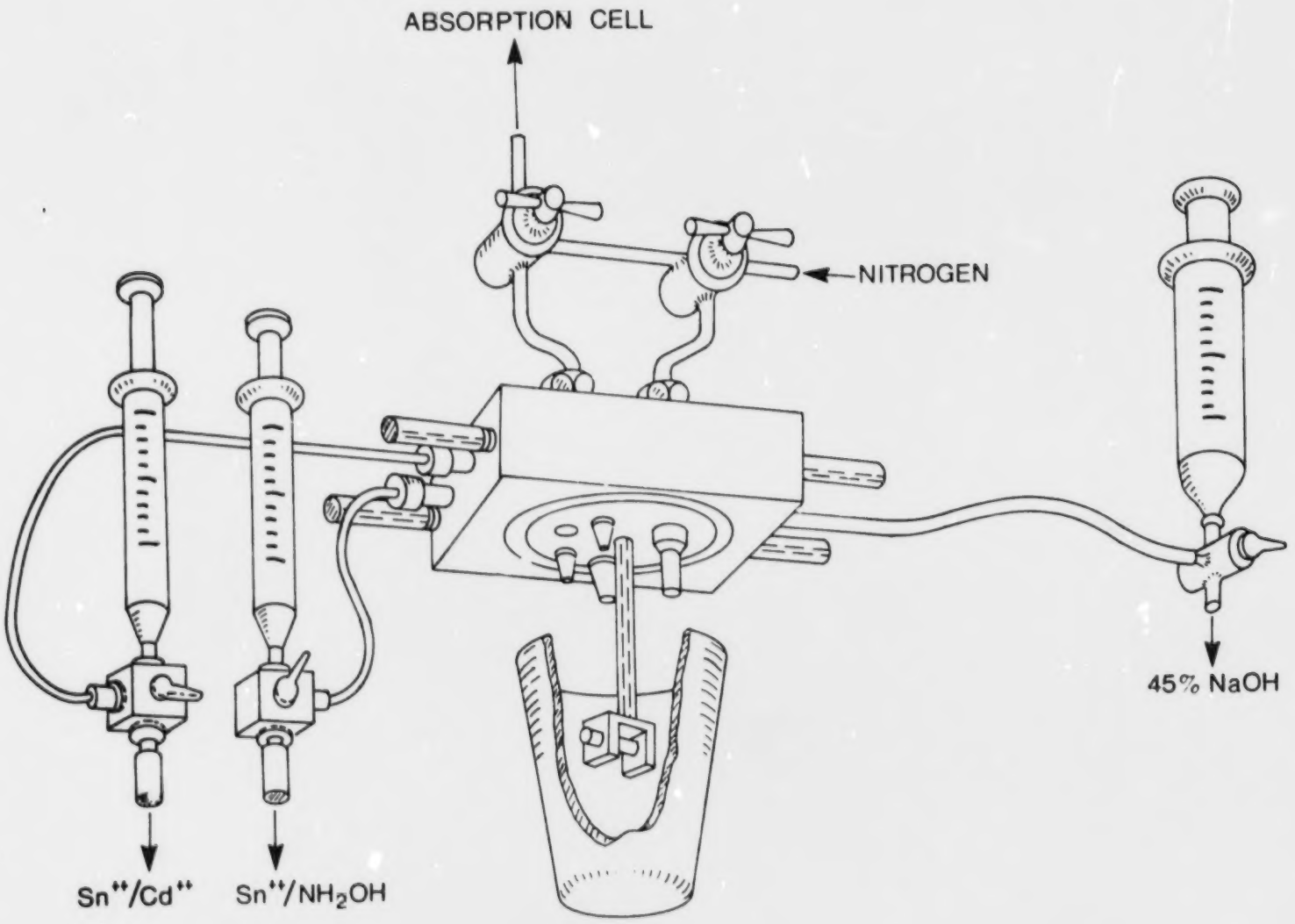

Ground Glass
Joint
24/40
Hook
154 mm
114 mm
Flat Bottom Tube
28 mm

Figure 3. Mercury reduction apparatus using disposable plastic cup.



Taken from Reference 10.2

Figure 4. Flow diagram for a typical mercury analysis.

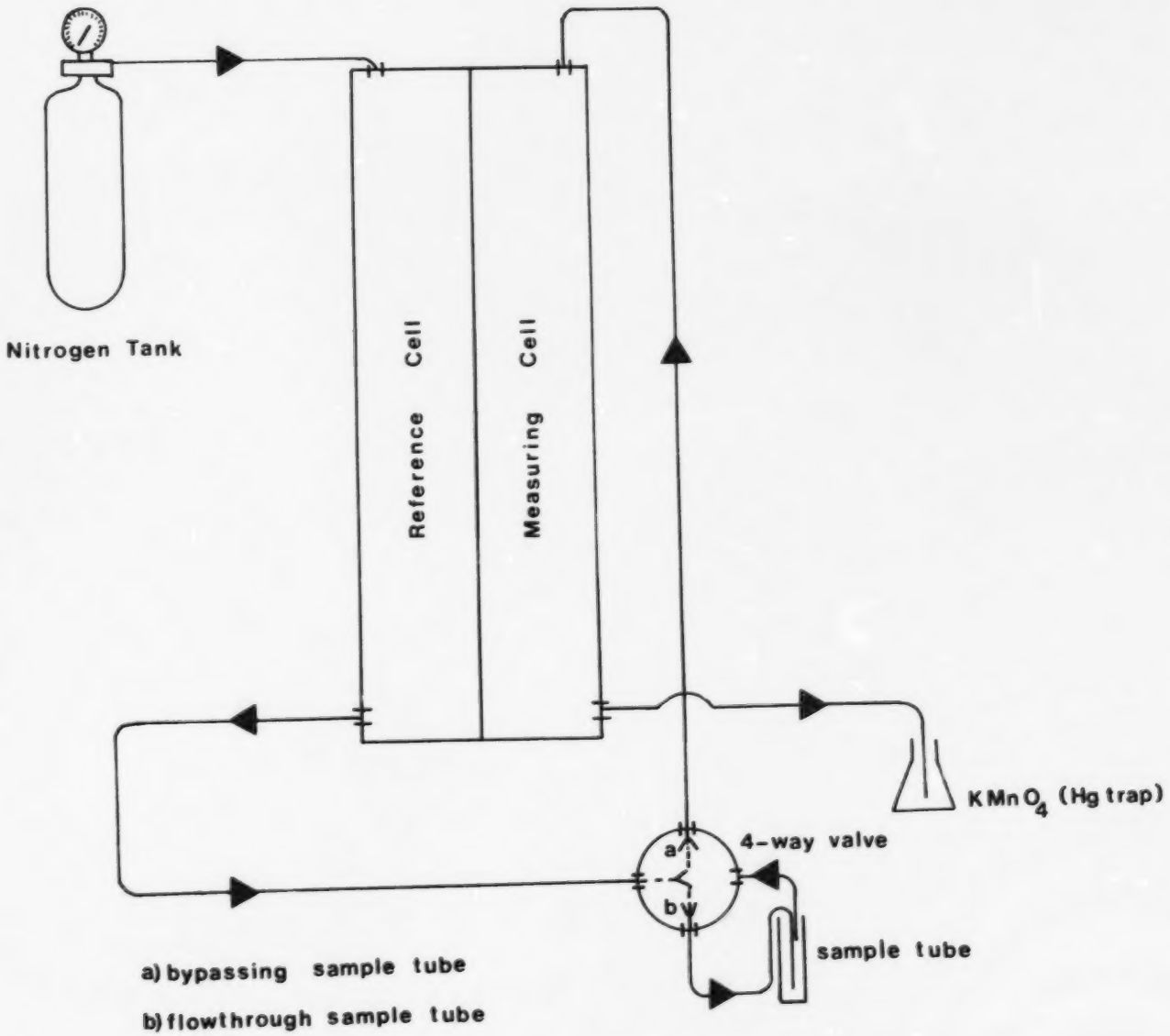
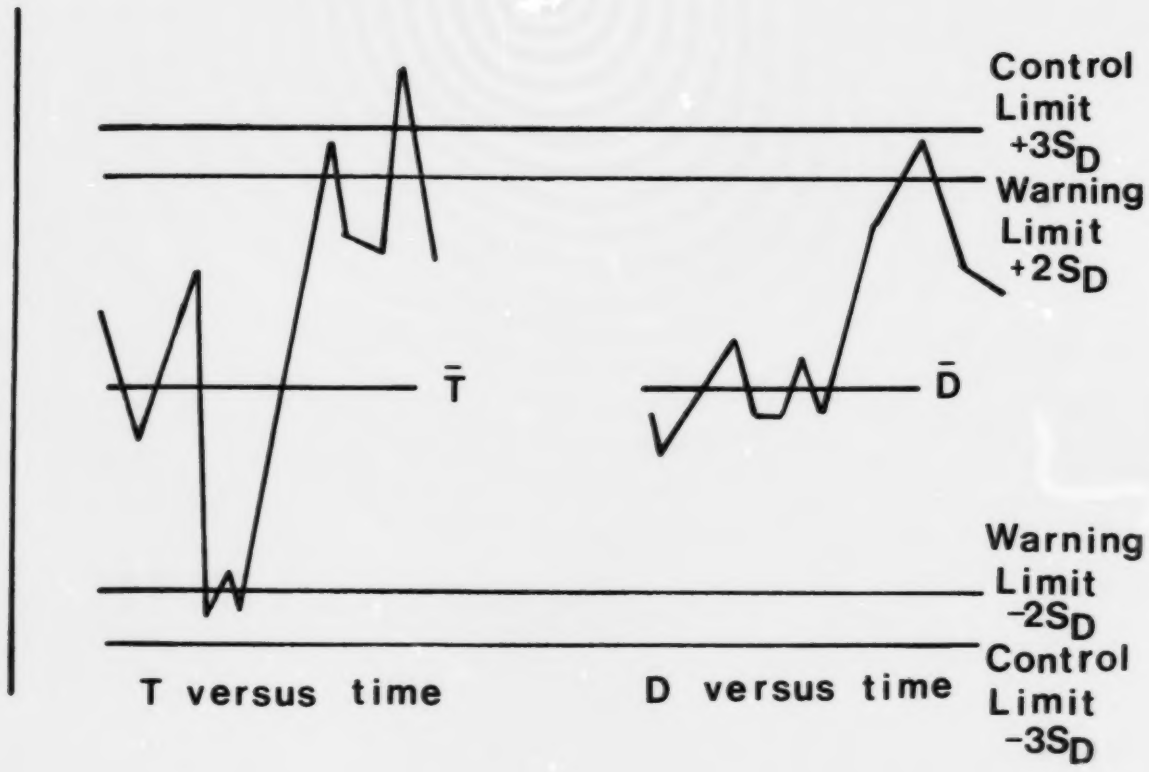

Nitrogen Tank
Reference Cell
Measuring Cell
KMnO4 (Hg trap)
4-way valve
a
b
sample tube
a) bypassing sample tube
b) flowthrough sample tube

Figure 5. Standard deviation of the Quality Control data and setting up of warning and control limits.


Control Limit +3S_D
Warning Limit +2S_D
$\bar{T}$
$\bar{D}$
Warning Limit −2S_D
Control Limit −3S_D
T versus time
D versus time

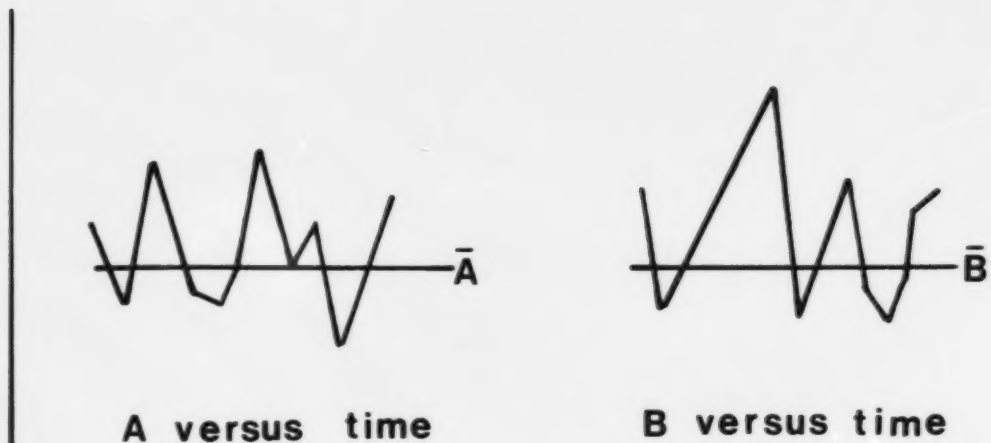
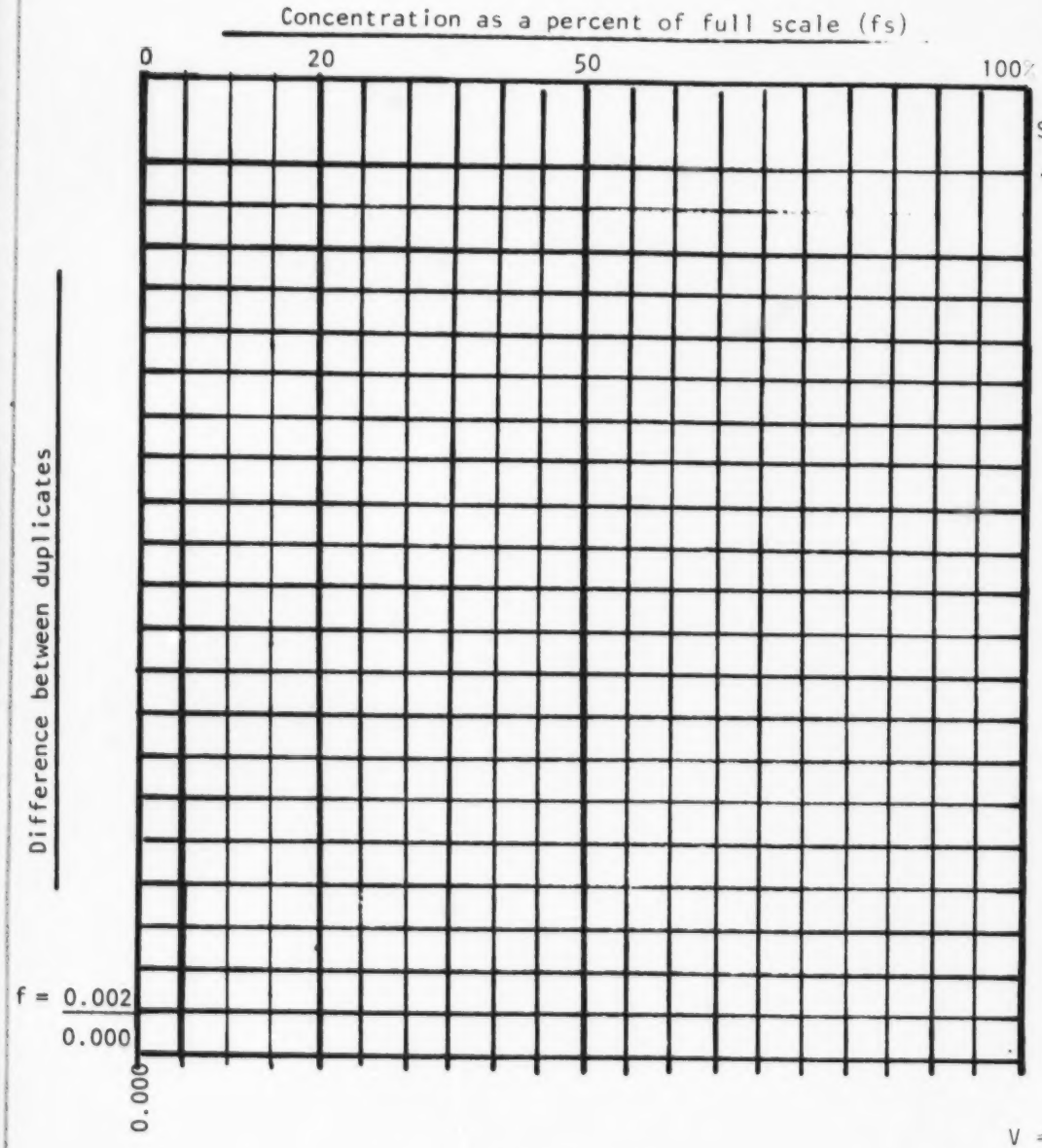

$\bar{A}$
$\bar{B}$
A versus time
B versus time

Figure 6. Evaluation of within-run precision: blank form.



(Taken from Ref. 10.4)

TABLE FOR CALCULATING STANDARD DEVIATION

Scaled D^2	0 - 20% fs		20 - 50% fs		50 - 100% fs	
	n	nD^2	n	nD^2	n	nD^2
400						
361						
324						
289						
256						
225						
196						
169						
144						
121						
100						
81						
64						
49						
36						
25						
16						
9						
4						
1						
0		0.		0.		0.
Σ						

$V = \Sigma nD^2/2\Sigma n$

$s = f\sqrt{V}$

Figure 7. Evaluation of within-run precision: data entry sheet.

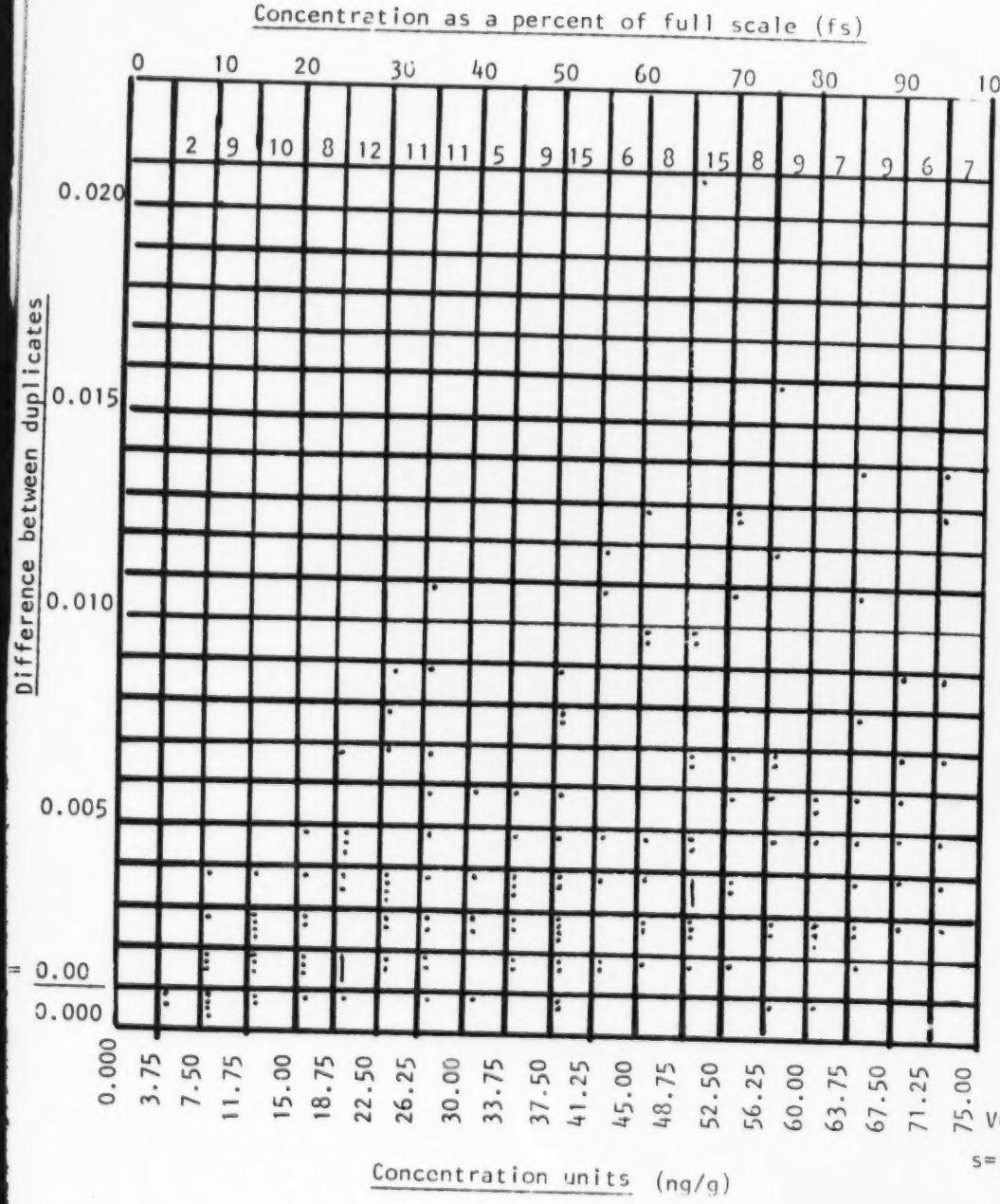


TABLE FOR CALCULATING STANDARD DEVIATION

Scaled D^2	0 - 20% fs		20 - 50% fs		50 - 100% fs	
	n	nD^2	n	nD^2	n	nD^2
400					1	400
361						
324						
289						
256						
225					1	225
196						
169					2	338
144					4	576
121					2	242
100			1	100	3	300
81					4	324
64			2	128	3	192
49			2	98	3	147
36			3	108	7	252
25			3	75	7	175
16			6	96	10	160
9	2	18	12	108	14	126
4	5	20	10	40	18	72
1	6	6	14	14	8	8
0	8	0	4	0	4	0
Σ	21	44	57	767	91	3537
$V=\Sigma nD^2/2\Sigma n$		1.0476		6.7281		19.4341
$s=f\sqrt{v}$		0.00102		0.0026		0.0044